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Network Pharmacology and Molecular Dynamics Simulation Study on the Mechanism of Wuda Jiangjun Ointment in Treating Chronic Lumbar Muscle Strain

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Abstract: *Objective*: To investigate the mechanism of Wuda Jiangjun Ointment in treating chronic lumbar muscle strain based on network pharmacology and molecular dynamics simulation. *Methods*: Active components of Wuda Jiangjun Ointment were identified using the TCMSP database, HERB database, and a review of relevant literature. SwissTarget Prediction, GeneCards, and OMIM databases were employed to predict targets related to chronic lumbar muscle strain. The intersection of drug and disease targets was obtained using the bioinformatics platform and imported into the STRING database to construct a protein-protein interaction (PPI) network. Cytoscape 3.10.2 software was used to visualize the PPI network and perform topological analysis to screen core targets. Functional enrichment analysis of GO and KEGG pathways for the intersecting targets was conducted using the DAVID database. A "drug-active component-disease-target" network diagram was constructed using Cytoscape 3.10.2. Molecular docking was performed using AutoDock software to simulate the binding of active components to target proteins. Furthermore, the Amber 24 software package was utilized to evaluate the binding stability between target proteins and active components. *Results*: A total of 75 active components and 350 corresponding potential targets of Wuda Jiangjun Ointment were screened, along with 2,159 disease targets for chronic lumbar muscle strain. There were 192 intersecting targets between the active components and the disease. Topological analysis of the PPI network identified TP53, AKT1, STAT3, etc., as core targets. Topological analysis of the "drug-active component-disease-target" network identified quercetin, dehydropipernonaline, tomentosin A, etc., as key components. GO and KEGG enrichment analyses indicated that the key pathways were primarily involved in the IL-17 signaling pathway, the AGE-RAGE signaling pathway in diabetic complications, and cellular senescence. Thus, the therapeutic mechanism was predicted to be related to inflammatory responses, among others. Molecular docking and molecular dynamics simulations verified that the core target proteins and active components exhibited good binding affinity. *Conclusion*: Wuda Jiangjun Ointment exerts anti-inflammatory effects and treats chronic lumbar muscle strain by participating in biological processes such as inflammatory responses through multiple components, targets, and pathways. This study provides a basis for further research into the molecular mechanisms.

Keywords: Wuda Jiangjun Ointment; Chronic lumbar muscle strain; Network pharmacology; Molecular dynamics simulation; Target prediction

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1. Introduction

Chronic lumbar muscle strain (CLMS) is a condition arising from prolonged overuse or chronic stress injury to soft tissues such as lumbar muscles, fascia, and ligaments, constituting one of the most common causes of chronic low back pain [1]. Clinically, it manifests as persistent dull pain or intermittent discomfort in one or both sides of the lumbar region, characterised by a protracted course prone to recurrent episodes [2]. Modern medical research identifies prolonged tension and fatigue within the lumbar musculature as primary risk factors for CLMS [3]. Pathologically, inflammatory mediators are involved throughout the process. Following acute or chronic injury to lumbar soft tissues, impaired local blood circulation disrupts the balance between tissue metabolic supply and demand. This leads to the accumulation of pathological metabolic by-products, triggering an aseptic inflammatory response that ultimately manifests as low back pain [4]. Traditional Chinese Medicine classifies this condition under categories such as “lumbar pain” and “muscle and tendon obstruction”[5]. The core pathogenesis in CLMS patients lies in qi stagnation and impaired blood circulation, obstructing the meridians. This obstruction causes pain, manifesting as lumbar discomfort [6]. Currently, Western medical approaches to treating CLMS primarily include non-steroidal anti-inflammatory drugs (NSAIDs), neurotrophic agents, analgesics, and physical rehabilitation therapies. While these methods effectively alleviate pain and improve function in the short term, prolonged medication use may induce adverse reactions such as gastrointestinal discomfort and hepatic or renal impairment [3]. Consequently, exploring safe, effective interventions with low recurrence rates has become a key focus in contemporary CLMS treatment research.

Traditional Chinese Medicine demonstrates notable efficacy in treating CLMS. Lifestyle factors, emotional stress, and environmental influences frequently disrupt the smooth flow of qi and blood in the lumbar region, rendering the qi stagnation and blood stasis pattern particularly prevalent in CLMS [7]. Wuda Jiangjun Ointment (WDJJO), an in-house formulation of Guangxi International Zhuang Medicine Hospital, comprises Polygonum cuspidatum, Curcuma zedoaria, Boswellia serrata, Drynaria fortunei, Polygonum aviculare, Ligusticum chuanxiong, Panax notoginseng, Piper longum, Dracocephalum moldavica, Mirabilite, Dragon’s Blood Resin, Borneol, and Fermented Black Bean with Ginger. This formulation synergistically promotes blood circulation, dispels stasis, relaxes tendons and activates meridians, regulates qi, and alleviates pain, thereby restoring smooth qi and blood flow, dissipating stagnation, and relieving discomfort [8]. Preliminary clinical observations indicate that Martial Arts General Ointment is widely employed in treating muscular pain, soft tissue injuries, osteoarthritis, rheumatoid arthritis, and neuralgia. It effectively promotes local blood circulation and improves stasis conditions, with its mechanism potentially involving inhibition of inflammatory responses and regulation of immune function. This study employs network pharmacology methods to predict the primary active constituents and potential mechanisms of action in Martial Arts General Ointment. Molecular docking and molecular dynamics simulations validate the binding affinity between these constituents and key targets, providing a theoretical foundation for further experimental research and clinical application.

2. Materials and methods

2.1. Screening of active ingredients and target prediction

Within the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) (https://www.tcmsp-e.com/), each herbal ingredient in Wudajunjiang Gao (Polygonum cuspidatum, Curcuma zedoaria, Boswellia serrata, Drynaria fortunei, Polygonum aviculare, Ligusticum chuanxiong, Panax notoginseng, and

Piper longum) as search keywords. Based on pharmacokinetic parameters and considering the topical application of this formulation, screening was conducted for active components with a drug-like property (DL) ≥ 0.18 to identify potential active constituents and their target sites. Concurrently, the HERB 2.0 database (https://www.herb.ac.cn/) and relevant literature were consulted to supplement the active components and target information for Chinese medicinal herbs not included in TCMSP (Feilongzhangxue, Mangxiao, Menthol, Longxuejie, Douchi Jiang). Finally, all candidate drug targets were input into the Uniprot database (https://www.uniprot.org/) for standardisation of their names into official gene symbols, facilitating subsequent network construction and functional analysis.

2.2. Disease target screening

Using "chronic lumbar muscle strain" as the search term, disease targets were obtained from the GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/) databases. After merging and deduping disease targets from each database, their names were standardised via the Uniprot database to obtain CLMS targets.

2.3. Identification of drug-disease intersection targets

Acquisition of Drug-Disease Intersection Target Database WDJJO and CLMS targets were imported into Venny 2.1.0 (https://bioinfogp.cnb.csic.es/) to identify common drug-disease targets, with a Venn diagram generated.

2.4. Construction of protein-protein interaction (PPI) network and core target screening

The intersection targets of WDJJO and CLMS were imported into the STRING database (https://cn.string-db.org/). Homo sapiens was selected as the study species, with the confidence threshold set to highest confidence ≥ 0.9. Unconnected nodes were hidden to generate the PPI network. Subsequently, the resulting PPI network was imported into Cytoscape 3.10.2 for visualisation analysis. The CytoNCA plugin was employed to calculate network topological parameters, including node degree, betweenness centrality, and closeness centrality. Core targets exhibiting key regulatory roles within the network were identified based on node degree values, with visualisation of the screening results.

2.5. Network construction and topological analysis

Construction and Analysis of the "Drug–Active Ingredient–Disease–Target" Network Diagram Using Cytoscape 3.10.2 software, we constructed an association network linking "Martial Arts General Ointment–Active Ingredient–Chronic Lumbar Muscle Strain–Target" to systematically reveal the potential key compounds responsible for the ointment's therapeutic efficacy. Subsequently, the CytoNCA plugin was employed to perform topological feature analysis on the network, calculating parameters including node degree, betweenness centrality, and closeness centrality. Primary active ingredients were evaluated based on their node degree values.

2.6. GO functional and KEGG pathway enrichment analysis

The selected target intersection was subjected to GO functional annotation and KEGG pathway enrichment analysis using the DAVID database (https://davidbioinformatics.nih.gov/). Results for biological processes (BP), cellular components (CC), and molecular functions (MF) were extracted alongside KEGG pathway outcomes, with $P < 0.05$ serving as the significance threshold. In GO analysis, the top 10 BP, CC, and MF entries with the smallest *P*-values were selected for enrichment display. For KEGG pathway analysis, signalling pathways with *P*

< 0.05 and strong association with CLMS were prioritised for investigation. Finally, enrichment bar charts for GO functions and KEGG pathways were generated using the MicroBioinformatics platform.

2.7. Molecular docking validation

The top three key active components by network analysis score and the top three core targets were selected for molecular docking. First, the three-dimensional structures (ligands) of the selected active components were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), while the corresponding three-dimensional crystal structures (receptors) of the core targets were obtained from the PDB database (https://www.rcsb.org/). Subsequently, the receptor proteins were pre-processed using PyMOL software to remove water molecules and redundant residues. AutoDockTools 1.5.7 was then employed to perform hydrogenation and charge optimisation on the ligands, converting both ligand and receptor files into pdbqt format. Following the configuration of Grid Box parameters based on the receptor active site, molecular docking calculations were conducted using AutoDock Vina[9]. Finally, PyMOL was employed for visualisation and presentation of the docking results.

2.8. Molecular dynamics simulation

The Amber 24 software package was utilised to assess the binding stability between proteins and compounds. The LEaP tool was employed for system construction: loading the PDB structures of the native protein and its mutants, applying the ff14SB force field to describe the protein, and employing the GAFF force field for compound parameterisation. The system was placed in a TIP3P water tank with a boundary distance of 10.0 Å from the protein surface. Na^+ and Cl^- ions were added to neutralise charges. To resolve structural conflicts, a two-stage energy minimisation was performed: first, constraints were applied to the protein backbone, optimising only the solvent and ions; subsequently, constraints were removed, and a full minimisation of the entire system was conducted. This combined the steepest descent method with the conjugate gradient method to ensure system stability. System equilibration occurred in two stages: initially, the system was heated from cryogenic temperatures to 300.0 K under isothermal conditions with the protein backbone constrained; subsequently, further equilibration under isobaric conditions adjusted density while maintaining 300.0 K and 1 bar pressure, progressively preparing the system for production runs. A 100 ns molecular dynamics simulation was run under isothermal and isobaric conditions at 300.0 K and 1 bar pressure, unconstrained, generating trajectories for subsequent analysis. The CPPTRAJ tool was employed to analyse the trajectories, calculating root mean square deviation (RMSD), radius of gyration (Rg), solvent-accessible surface area (SASA), root mean square fluctuation (RMSF), and hydrogen bond count. The MM/GBSA method was employed to estimate binding free energy. One hundred frames were extracted from the final 1 ns of the 100 ns trajectory to analyse van der Waals forces, electrostatic interactions, and polar and non-polar solvation contributions, expressed in kJ/mol.

3. Results

3.1. Active components and target screening results

Compounds from each medicinal ingredient of WDJJO were retrieved from the TCMSP database. Based on the requirement DL ≥ 0.18, 75 chemical components were preliminarily screened: 10 from Polygonum cuspidatum, 18 from Drynaria fortunei, 15 from Piper longum, 8 from Panax notoginseng, 7 from Ligusticum chuanxiong, 8

from Boswellia serrata, 6 from Schizonepeta tenuifolia, Curcuma zedoaria 3 (see **Table 1**). This yielded 1,136 target genes for the identified active components, which were deduplicated to obtain 270 valid targets. As the TCMSP database did not include the five medicinal ingredients Feilongzhangxue, Mangxiao, menthol, dragon's blood resin, and fermented black beans. These five herbs were identified via the HERB 2.0 database, literature searches, and retrieval of their canonical SMILES sequences from the PubChem database. Target predictions for these compounds were then generated using the Swiss Target Prediction online database. After deduplication of the predicted results, 80 targets were collected. Following consolidation, the total number of targets reached 350.

Table 1. Number of active components and targets of major Chinese medicinal herbs in Wuda Jiangjun Ointment

Chinese Medicinal Herb	Number of Active Components	Number of Targets
Polygonum cuspidatum	10	324
Drynaria fortunei	18	304
Piper longum	15	157
Ligusticum chuanxiong	7	42
Cynanchum paniculatum	6	16
Curcuma phaeocaulis	3	24
Panax notoginseng	8	253
Boswellia carterii	8	16

3.2. Acquisition of disease targets

A total of 2,000 and 200 CLMS targets were retrieved and screened from the GeneCards and OMIM databases respectively. Following data consolidation and deduplication, 2,159 relevant targets were obtained.

3.3. Acquisition of intersecting drug-disease targets

CLMS targets and WDJJO targets were input into the Venny 2.1.0 online diagramming platform, yielding 192 intersecting targets (see **Figure 1**).

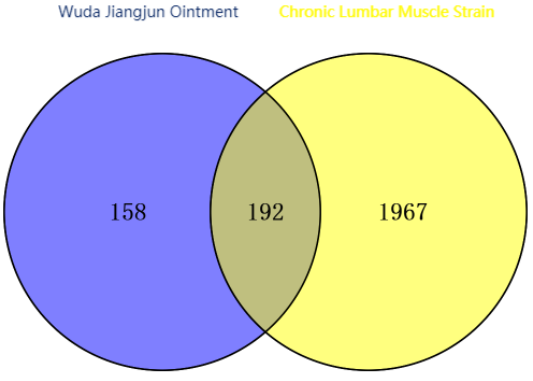


Figure 1. Venn diagram of target points for "Martial Arts General Ointment - Chronic Lumbar Muscle Strain"

3.4. Construction of PPI network and core target screening

The 192 intersecting targets were imported into the STRING protein interaction analysis platform, with species restricted to humans. Interactions with a protein interaction score ≥0.9 were selected, yielding the PPI relationship diagram (see **Figure 2A**). In **Figure 2A**, 191 nodes and 707 edges are visible, with each node representing a target protein. Protein-protein interactions are depicted as straight line connections; higher network connectivity density indicates closer relationships between proteins, providing a theoretical basis for further core target screening. The PPI network diagram was visualised using Cytoscape 3.10.2 software (see **Figure 2B**). where node size and colour intensity correspond to degree values—larger nodes and darker hues denote higher degrees. Concurrently, the top 10 core target nodes by degree were selected and plotted (**Figure 2C**), indicating these 10 targets as potential therapeutic candidates for WDJJO in chronic LMS treatment: TP53, AKT1, STAT3, JUN, ESR1, SRC, TNF, IL6, NFKB1, and HSP90AB1. Detailed parameters are presented in **Table 2**.

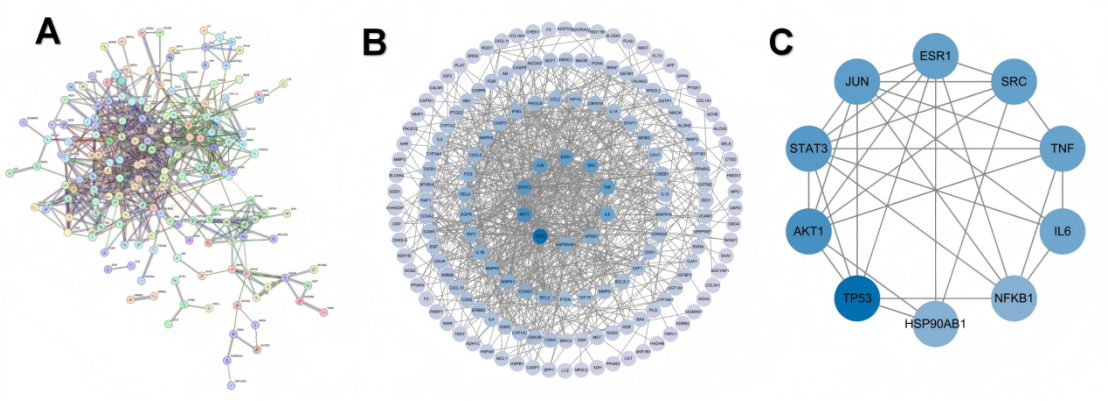


Figure 2. PPI network diagram of intersecting targets for "martial arts general ointment - chronic lumbar muscle strain"

Table 2. Topological analysis results of core target networks for "Martial Arts General Ointment - Chronic Lumbar Muscle Strain"

Target	Degree
TP53	54
AKT1	38
STAT3	34
JUN	32
ESR1	31
SRC	31
TNF	30
IL6	28
NFKB1	22
HSP90AB1	22

3.5. Construction and analysis of the "drug–active ingredient–disease–target" network diagram

The relationship network between "drug–active ingredient–disease–target" was visualised and analysed using

Cytoscape 3.10.2 software (see **Figure 3**). Red rectangles denote disease names, blue rectangles represent drug names, green rectangles indicate active ingredients, yellow rectangles denote pathway names, and purple rectangles signify shared targets. Node size in the network diagram is proportional to Degree values. The CytoNCA plugin within the software analysed the topological parameters of the active ingredient network. Results indicated that quercetin (MOL000098), dehydropipernonaline (MOL001561), and Tomentolide A (MOL005619) exhibit higher Degree values, indicating these components are the primary active constituents in WDJJO's treatment of CLMS.

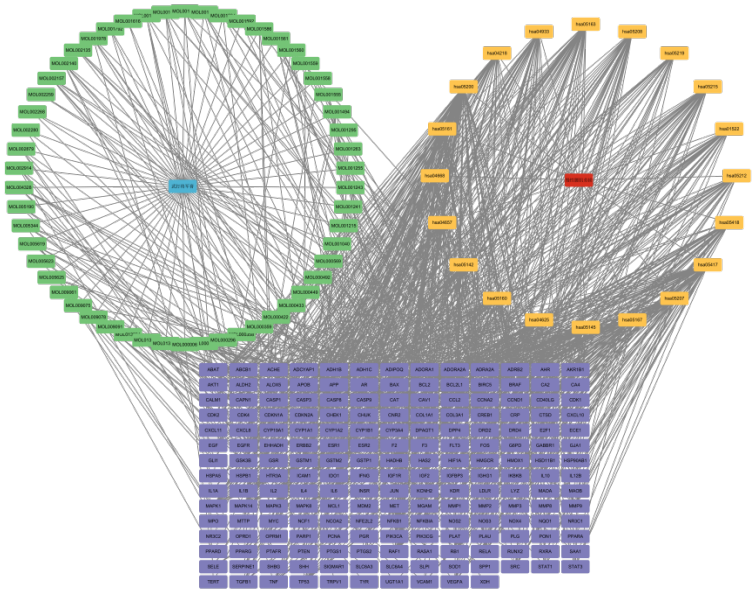


Figure 3. Network diagram illustrating the relationship between drugs, active ingredients, diseases, and targets

3.6. Gene ontology analysis and KEGG pathway enrichment analysis

The intersection targets of WDJJO treating CLMS were imported into the DAVID database for Gene Ontology (GO) analysis and KEGG pathway analysis, with data collected. Using $P < 0.05$ as the threshold, 736 entries for BP, 190 entries for MF, and 86 entries for CC were collected. The 10 entries with the smallest *P*-values from the BP, CC, and MF results were selected for enrichment analysis, and a GO enrichment bar chart was plotted (see **Figure 4**). Results indicate that WDJJO treatment of CLMS primarily enriches biological processes including positive regulation of gene expression, negative regulation of apoptosis, positive regulation of cell population growth, and positive regulation of microRNA transcription; tissue structures such as cytoplasm, plasma membrane, membrane raft, and receptor complex; and molecular functions including identical protein binding, enzyme binding, transcription coactivator binding, and nuclear receptor activity.

In the KEGG pathway enrichment analysis, 176 pathways were identified using a $P < 0.05$ threshold. The top 20 enriched pathways are presented in a KEGG pathway enrichment bubble plot (**Figure 5**). Results indicate that after excluding pathways unrelated to chronic lumbar muscle strain (e.g., cancer pathways, prostate cancer, toxoplasmosis), Wudajunjiang ointment treatment exhibited the most pronounced enrichment in inflammation-related metabolic pathways. Additionally, relevant pathways for WDJJO treatment of CLMS showed significant enrichment in IL-17 signalling pathways, AGE-RAGE signalling pathways in diabetic complications, and cellular senescence signalling pathways.

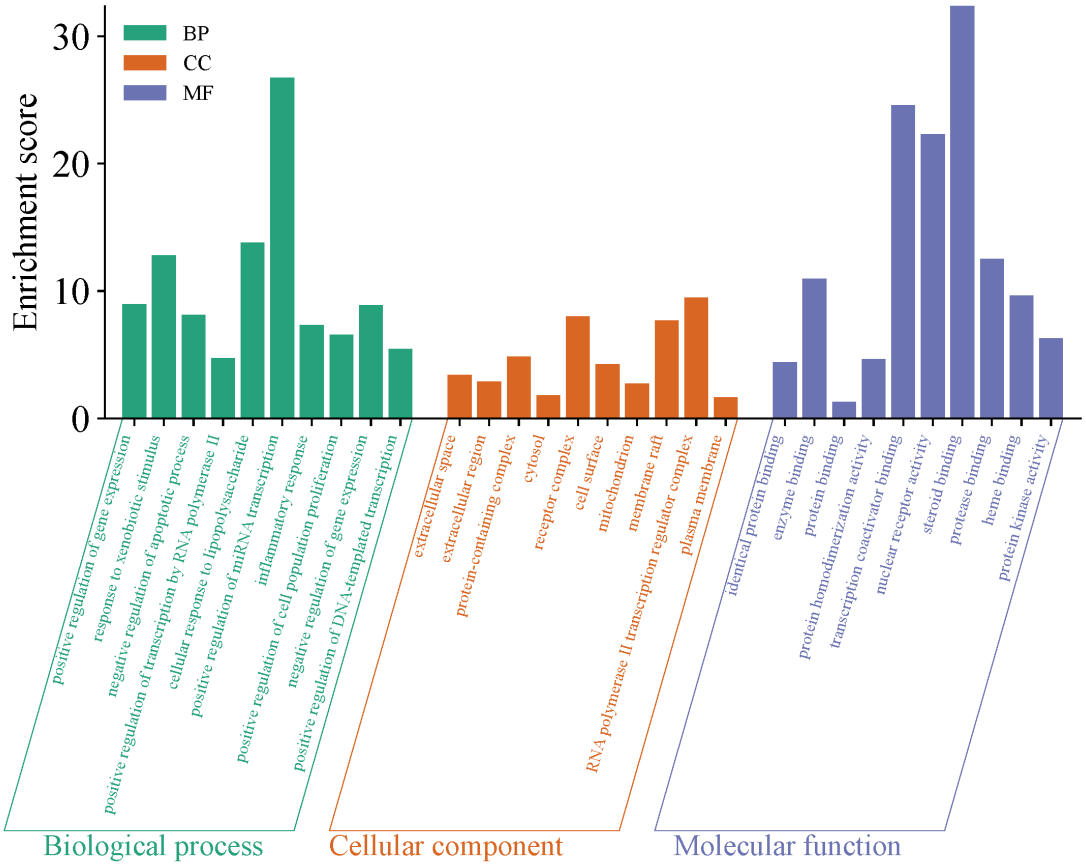


Figure 4. GO enrichment diagram for "Martial Arts General Ointment - Chronic Lumbar Muscle Strain"

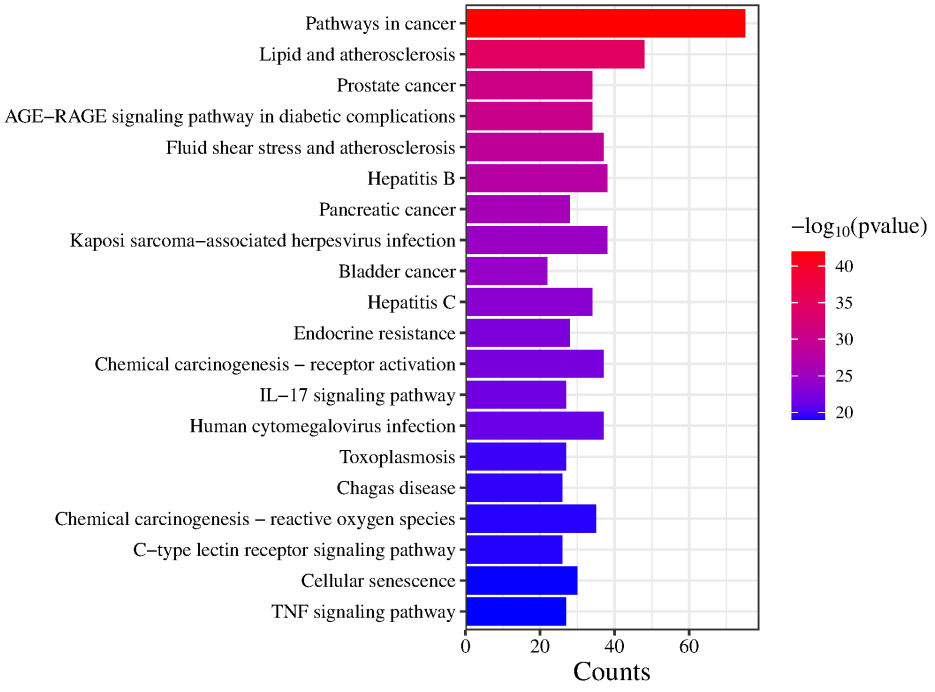


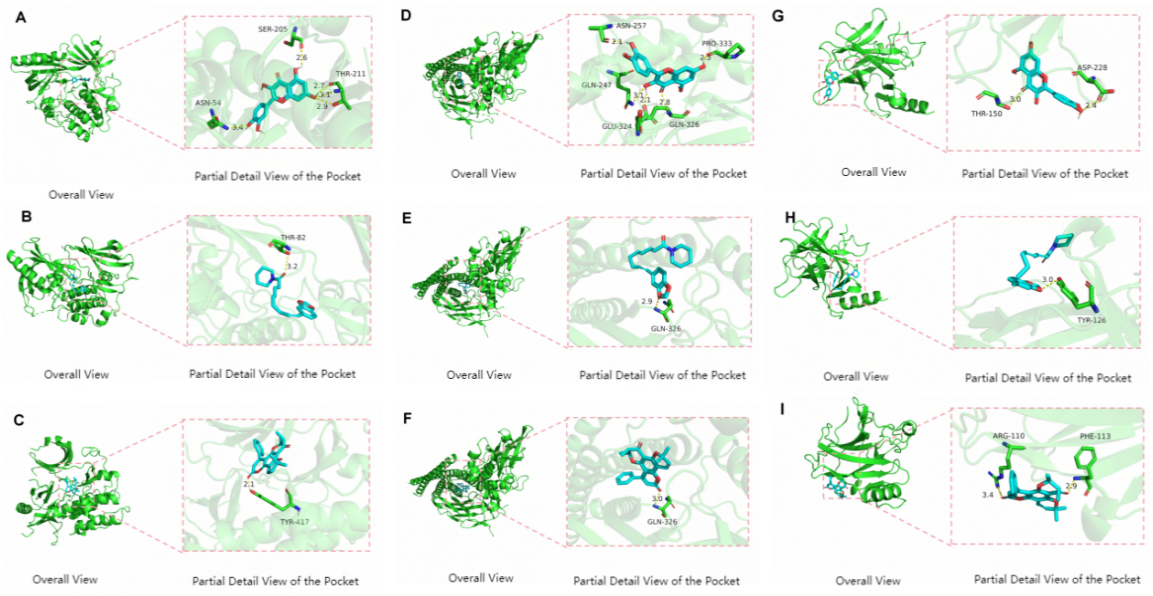
Figure 5. KEGG pathway enrichment diagram for "Martial Arts General Ointment - Chronic Lumbar Muscle Strain"

3.7. Molecular docking

To further validate the network pharmacology findings, the top three key active components with the highest degrees from the "drug-active ingredient-disease-target" network relationship diagram were selected as pharmacodynamic molecules. The top three core targets identified via PPI analysis—TP53, AKT1, and STAT3 as docking targets. Their binding energies were determined, yielding nine sets of docking results as shown in **Table 3**. Generally, docking energies below -4.25 kcal/mol indicate some binding activity, values below -5.0 kcal/mol denote favourable binding activity, while those below -6.0 kcal/mol signify strong binding activity [10]. The results demonstrate that the minimum binding energies for all compounds with the protein were below -4.25 kcal/mol, with 100% of compounds exhibiting binding energies ≤ -5.0 kcal/mol. These findings indicate that the key active components of WDJJO predicted in this study exhibit favourable binding activity with critical therapeutic targets for CLMS, validating the scientific rigour and reliability of the network pharmacology predictions. Notably, the key component quercetin exhibited binding energies < -6.0 kcal/mol with TP53, AKT1, and STAT3; dehydropipronalin with AKT1 and STAT3; and villus lactone A with TP53, AKT1, and STAT3, indicating favourable binding affinity. Molecular docking results were visualised using PyMOL software, as depicted in **Figure 6**.

Table 3. Docking results of proteins and ligands

Protein	Ligand	Binding Energy (kcal/mol)
AKT1	Quercetin	-9.8
AKT1	Dehydropipernonaline	-9.9
AKT1	Tomentolide A	-10.0
STAT3	Quercetin	-7.4
STAT3	Dehydropipernonaline	-6.9
STAT3	Tomentolide A	-8.1
TP53	Quercetin	-6.9
TP53	Dehydropipernonaline	-5.6
TP53	Tomentolide A	-7.7



Note: A: AKT1 and quercetin; B: AKT1 and dehydropipronalin; C: AKT1 and villus lactone A; D: STAT3 and quercetin; E: STAT3 and dehydropipronalin; F: STAT3 and villus lactone A; G: TP53 and quercetin; H: TP53 with dehydropipronalin; I: TP53 with vellin A

Figure 6. Visualisation of molecular docking results for core components of Wuda Jiangjun ointment and their binding energies with key target proteins

3.8. Molecular dynamics simulation

Molecular dynamics simulations were conducted using AKT1 and quercetin (MOL000098). The results are as follows:

3.8.1. Hydrogen bond analysis

To investigate the hydrogen bonding properties at the binding site of the AKT1-quercetin complex, we calculated the number of primary hydrogen bonds stabilising the interaction between AKT1 and quercetin. The results indicate that the number of hydrogen bonds between AKT1 and quercetin fluctuates minimally, remaining stable between 0 and 1 throughout the simulation. This suggests a relatively stable hydrophilic binding interaction between the two molecules (see **Figure 7A**).

3.8.2. RMSD analysis

Root mean square deviation (RMSD) serves as a crucial metric for assessing the stability of protein-ligand complexes. A flatter RMSD curve indicates greater complex stability. Results show that the RMSD curve for the AKT1-quercetin complex fluctuates within approximately 2 Å without significant variation, indicating high stability (see **Figure 7B**).

3.8.3. RMSF analysis

Root mean square fluctuation (RMSF) reflects the degree of motion exhibited by amino acid residues within a protein during simulation. Higher RMSF values indicate greater residue fluctuations, while lower RMSF values suggest reduced residue motion (see **Figure 7C**).

3.8.4. SASA analysis

Solvent-accessible surface area (SASA) measures the contact area between a protein surface and the solvent, serving as a crucial parameter for studying protein stability, interactions, and folding. Hydrophobic interactions constitute the primary driving force for protein folding, whereas regions in contact with the solvent are typically polar and exhibit weaker hydrophobic interactions. Results demonstrate a marked increase in SASA values around 10 ns and 60 ns, indicating enhanced interactions between the molecular surface and solvent. This may influence solubility, reactivity, and biological activity (see **Figure 7D**).

3.8.5. Rg analysis

The radius of gyration (Rg) is a physical quantity describing the compactness of a protein structure; a smaller Rg indicates a more compact and stable structure. The Rg curve calculated from molecular dynamics simulations shows that the Rg of the AKT1-quercetin complex remained stable throughout the simulation with minimal fluctuations, consistent with the stability of the RMSD curve. Minor fluctuations observed towards the simulation tail (around 60 ns) corroborated SASA analysis results, indicating overall good stability of the complex (see **Figure 7E**).

3.8.6. MM/GBSA analysis

The MM/GBSA (Molecular Mechanics/Generalised Born Surface Area) method is a widely employed computational approach for estimating the binding free energy of protein-compound complexes, thereby assessing

binding affinity and stability. In this study, 100 frames (one frame every 10 ps) were extracted from the final 1 ns of a 100 ns molecular dynamics trajectory for analysis. The binding free energy (ΔG_binding) was estimated by comparing the free energy differences between the AKT1-quercetin complex, the receptor, and the ligand, as shown in **Table 4**.

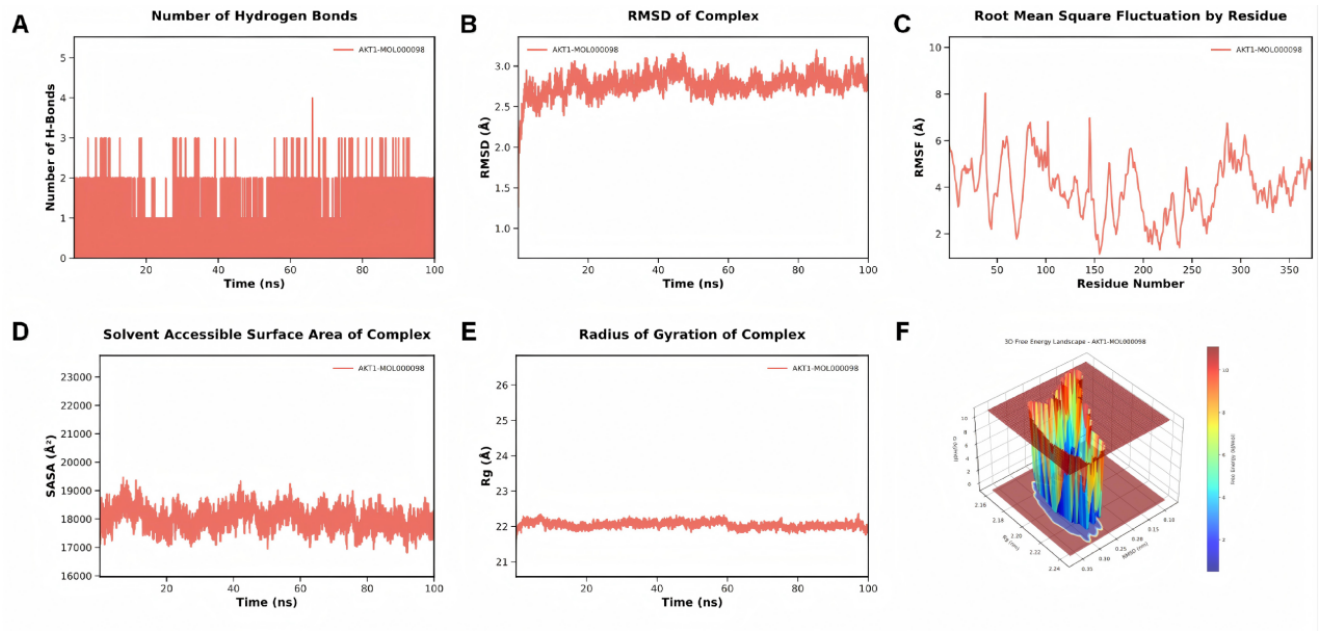
Table 4. Free energy distribution of AKT1-quercetin (MOL000098: quercetin) complex

Energy Type	Compound (kJ/mol)
E_VDW: Van der Waals Energy	-173.1348±8.4077
E_ELE: Electrostatic Energy	-51.0975±11.1943
E_GB: Polar Solvation Contribution	115.2930±10.6822
E_SA: Non-Polar Solvation Contribution	-21.6438±0.7301
ΔG_binding: Binding Free Energy	-130.5835±9.2492

3.8.7. Free energy landscape analysis

By plotting the free energy distribution of the AKT1-quercetin complex in a two-dimensional space of root mean square deviation (RMSD) and radius of gyration (Rg) during molecular dynamics simulations, the conformational stability and dynamic behaviour of the complex were revealed. In the figure, RMSD indicates the deviation of the complex from its initial structure, Rg reflects the compactness of the molecular structure, and free energy (G, unit: kJ/mol) quantifies the thermodynamic stability of each conformational state. Low-free-energy regions (deep blue) correspond to stable conformations with high probability, typically representing energy troughs of the complex; high-free-energy regions (red) correspond to unstable transition states or low-probability conformations. Contour lines in the bottom-up projection further highlight densely populated regions of the free energy distribution, aiding identification of major conformational clusters and their distribution patterns. By analysing the free energy landscape, this study identified stable conformations of the complex within specific RMSD and Rg ranges, providing crucial thermodynamic insights into its binding mechanism and structural dynamics (see **Figure 7F**).

Molecular dynamics simulations and subsequent analysis demonstrated high stability for the AKT1-quercetin complex. RMSD and Rg analysis revealed minimal structural fluctuations and a compact complex architecture; RMSF indicated stable amino acid residue motion; SASA analysis revealed solvent interaction changes; hydrogen bond analysis confirmed the stability of hydrophilic interactions; MM/GBSA analysis further quantified the binding free energy, indicating that van der Waals forces and electrostatic interactions are the primary drivers of complex stability. These findings provide profound insights into the binding mechanism of the AKT1-quercetin complex, offering a theoretical basis for subsequent drug design and optimisation.



Note: A: Hydrogen bond count curve; B: RMSD curve; C: RMSF curve; D: SASA curve; E: Rg curve; F: Free energy landscape plot

Figure 7. Molecular dynamics simulation results for AKT1 and quercetin (MOL000098)

4. Discussion

Chronic lumbar muscle strain predominantly affects middle-aged and elderly individuals, manifesting primarily as recurrent aching pain in the lumbosacral region, with symptoms exacerbated by physical activity or climatic changes. The widespread prevalence of sedentary office work has led to prolonged static tension in muscle groups, resulting in an increasing incidence of this condition [11]. Its aetiology is closely associated with poor posture, repetitive lumbar loading, chronic cumulative injury, and incomplete recovery from acute trauma [12]. Persistent mechanical stress induces microdamage to soft tissues and local ischaemia, leading to metabolic dysfunction, retention of metabolic by-products, oedema, and aseptic inflammation, thereby triggering or perpetuating pain. The core mechanism involves chronic strain of lumbar soft tissues and disruption of the local microenvironment [13].

The Martial Arts General Ointment, an in-house preparation at Guangxi International Zhuang Medicine Hospital, has been utilised for many years. Within the formula, Polygonum cuspidatum possesses effects of promoting blood circulation to remove stasis, unblocking meridians to alleviate pain, and clearing heat to drain dampness; Curcuma zedoaria both regulates qi to relieve pain and breaks up blood stasis to disperse nodules; Xu Changqing is renowned for dispelling wind and alleviating pain. Together, these three herbs both invigorate blood circulation to remove stasis and regulate qi to relieve pain, addressing both symptoms and root causes, thus serving as the principal herbs. Chuanxiong, Feilongzhangxue, and Bi Ba complement the principal herbs by enhancing their effects in invigorating blood circulation to remove stasis, reducing swelling, and alleviating pain, collectively functioning as the auxiliary herbs. Dipsacus asper fortifies the kidneys, strengthens bones, and promotes blood circulation to heal injuries. Boswellia, Panax notoginseng, and Dracaena draco all invigorate blood circulation, unblock meridians, alleviate pain, and reduce swelling. Piper longum and fermented black beans, with

their pungent and warm nature, dispel cold, regulate qi, and expel wind, serving as auxiliary herbs to enhance the formula's efficacy in dispelling wind, unblocking meridians, and relieving pain. Menthol and Epsom salt serve as guiding agents: the former, aromatic and cooling, clears orifices and alleviates pain; the latter, cold and salty in nature, expels heat pathogens, reduces swelling, and disperses nodules. Together, they direct the formula into the meridians, harmonise the properties of the herbs, and enhance the overall therapeutic efficacy. The formula employs both cold and warm properties, combining tonification with dispersion. It invigorates blood circulation without harming the vital energy, removes stasis while alleviating pain, embodying the therapeutic principle that "unblocking relieves pain, and invigoration prevents stiffness".

Through the "medicine–active ingredient–disease–target" network analysis, the core components of Wu Da Jiang Jun Plaster for treating chronic lumbar muscle strain were identified as quercetin, dehydrated piperin nanol, and villus lactone A. Quercetin, a widely distributed flavonoid compound, exhibits multifaceted effects including anti-inflammatory, antioxidant, and anti-fibrotic properties [14]. Its potential mechanisms may involve regulating the nuclear factor kappa-B (NF-κB) signalling pathway, thereby inhibiting the release of pro-inflammatory cytokines (such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6)) and alleviating local aseptic inflammation [15]. Furthermore, as a potent antioxidant, quercetin scavenges excess reactive oxygen species (ROS), mitigating oxidative stress-induced damage to muscle cells and surrounding tissues [16]. Research indicates quercetin inhibits fibroblast differentiation into myofibroblasts and reduces synthesis of type I and III collagen, suggesting potential value in alleviating muscle tissue fibrosis during the late stages of chronic lumbar muscle strain [17]. Dehydropiparone is an amide-type alkaloid whose mechanism of action is primarily hypothesised to involve analgesic and anti-inflammatory effects. Research into the mechanism of dehydrated piperine nonalin in treating chronic lumbar muscle strain remains limited. However, based on its structural characteristics and studies of similar compounds, it is hypothesised that it may exert analgesic effects on the central or peripheral nervous system by influencing ion channels such as the transient receptor potential vanilloid type 1 (TRPV1) or voltage-gated sodium channels, thereby alleviating chronic pain induced by lumbar muscle strain [18]. Visceral lactone A is a structurally unique sesquiterpene compound exhibiting anti-inflammatory and cytoprotective effects [19]. It may reduce inflammatory mediator production by inhibiting macrophage activation or blocking the mitogen-activated protein kinase (MAPK) signalling pathway, thereby mitigating inflammatory damage to muscle tissue [20].

To determine the interrelationship of shared targets in Wu Da Jiangjun Ointment's treatment of chronic lumbar muscle strain, PPI topological analysis across diverse databases identified the top three key genes: TP53, AKT1, and STAT3. TP53, fully termed tumour protein 53, is fundamentally a transcription factor and one of the most crucial tumour suppressor genes within cells [21]. It primarily regulates key biological processes including cell cycle arrest, DNA repair, cellular senescence, and apoptosis, playing a central role in maintaining genomic stability and intracellular homeostasis. The pathogenesis of chronic lumbar muscle strain involves chronic injury, inflammation, and abnormal remodelling of muscle and connective tissue cells. Research suggests that under conditions of muscle tissue injury or stress, TP53 activation may drive damaged cells towards apoptosis or senescence. While TP53-mediated apoptosis may aid in clearing severely damaged myocytes, excessive activation could lead to chronic muscle fibre loss and inadequate repair. Furthermore, the TP53-regulated cellular senescence process may induce the secretion of pro-inflammatory cytokines by surrounding tissues, thereby exacerbating chronic inflammation. This accelerates the pathological progression of lumbar muscle strain and impairs long-term functional recovery of the affected tissue [22]. AKT1 is a pivotal member of the AKT serine/threonine protein kinase family and a key intracellular signalling molecule. It exerts central regulatory roles in cellular survival,

proliferation, metabolism, growth, and apoptosis [23]. In chronic lumbar muscle strain, muscle tissue remains in a state of persistent stress and imbalanced repair. The AKT1 signalling pathway is recognised as a key pathway for muscle hypertrophy and growth, with its activity being crucial for muscle tissue regeneration and repair. Moderate AKT1 activity exerts a protective effect on the repair and regeneration of injured muscle, promoting myocyte survival, inhibiting apoptosis, and potentially regulating the balance between protein synthesis and degradation in muscle fibres. However, under certain chronic inflammatory or ischaemic stress conditions, abnormal or prolonged suppression of AKT1 signalling may lead to muscle atrophy and diminished repair capacity, exacerbating the pathophysiological process of chronic lumbar muscle strain. This manifests as reduced muscle strength and persistent, unresolved pain [24]. STAT3, fully termed Signal Transduction and Activation of Transcription 3, is essentially a transcription factor mediating the signal transduction of various cytokines (such as IL-6, IL-10, etc.) and growth factors. It participates in regulating processes including inflammation, immune responses, cell proliferation, and differentiation [25]. Inflammation constitutes a pivotal component in the pathogenesis of chronic lumbar muscle strain. As STAT3 serves as a key downstream signalling molecule for the pro-inflammatory cytokine IL-6, it plays a significant role in mediating chronic inflammatory responses within lumbar muscle tissue. Within the pathological environment of chronic lumbar muscle strain, sustained STAT3 activation may promote the expression of pro-inflammatory factors, establishing a vicious cycle of inflammation that continuously stimulates pain receptors. It may also influence fibroblast activity, potentially promoting fibrosis, leading to reduced muscle flexibility and scar formation, thereby further restricting movement and inducing chronic pain [26].

GO analysis indicates that Wuda Jiangjun Ointment exerts specific effects in positively regulating gene expression, negatively regulating apoptosis, positively regulating cell proliferation, and influencing cytoplasmic, plasma membrane, enzyme-binding, and nuclear receptor activities. KEGG analysis indicates that Wuda Jiangjun Ointment may exert therapeutic effects on chronic lumbar muscle strain through inflammation-related metabolic pathways, IL-17 signalling pathways, AGE-RAGE signalling pathways in diabetic complications, cellular senescence, and other mechanisms. Relevant core targets also participate in multiple signalling pathways including PI3K/AKT and JAK/STAT. The IL-17 signalling pathway plays a pivotal role in the chronic inflammation of lumbar muscle strain. Upon binding to its receptor, IL-17 activates the ACT1-TRAF6 complex, subsequently initiating the NF-κB and MAPK pathways to induce substantial expression of downstream inflammatory mediators (IL-6, TNF-α, IL-1β) [27]. Research indicates that IL-17 signalling can upregulate the TP53 stress response pathway, promoting cell cycle arrest and cellular senescence in injured tissues. Concurrently, by enhancing STAT3 phosphorylation, it sustains the chronic inflammatory microenvironment. Abnormal activation of this pathway may prolong inflammatory responses in muscle and fascial tissues while diminishing repair capacity, thereby propelling lumbar muscle strain towards chronicity [28]. The PI3K/AKT pathway constitutes a crucial signalling axis regulating muscle cell survival, energy metabolism, and regeneration. Its core molecule, AKT1, plays a pivotal role in tissue repair during chronic lumbar muscle strain. PI3K activation promotes AKT1 phosphorylation; activated AKT1 inhibits myofibroblast apoptosis while promoting cell proliferation and maintaining metabolic homeostasis. Imbalance in this pathway may result in inadequate regeneration, metabolic disruption, and chronic pain [29]. The JAK/STAT pathway holds significant importance in chronic inflammation and tissue repair mechanisms, with STAT3 serving as a pivotal transcription factor. In chronic lumbar muscle strain, cytokines such as IL-6 activate JAK kinases, leading to STAT3 phosphorylation and nuclear translocation. This regulates the expression of genes associated with inflammatory responses, apoptosis, and fibrosis. Persistently activated STAT3 promotes fibroblast proliferation and collagen deposition, leading to fascial adhesions and tissue stiffness. Concurrently, it interacts

with TP53 signaling to regulate the balance between cellular stress and apoptosis[30]. This pathway plays a central role in sustaining chronic inflammation and tissue structural remodelling, constituting a key molecular target for chronic lumbar muscle strain.

Molecular docking results indicate that all compound pairs exhibit minimum binding energies below -5.0 kcal/mol, demonstrating high binding efficiency and strong affinity. Results show that quercetin, dehydropipronalin, and villus lactone A form stable docking models with TP53, AKT1, and STAT3, respectively. However, it should be noted that molecular docking outcomes may not always accurately reflect the most precise docking configuration. To address this limitation, MD simulations were conducted on the AKT1-quercetin complex system. Through various analyses, further evidence was obtained supporting the stability and strong binding between AKT1 and quercetin. Consequently, molecular docking and molecular dynamics simulation results indicate that Wuda Jiangjun Ointment exhibits stable binding with core targets, potentially exerting therapeutic effects on chronic lumbar muscle strain.

5. Conclusion

In summary, this study employed network pharmacology and molecular dynamics simulation to predict the potential mechanism by which Wuda Jiangjun Ointment treats chronic lumbar muscle strain through multi-component, multi-target, and multi-pathway interactions. This provides a theoretical foundation for its clinical application in treating chronic lumbar muscle strain and underpins subsequent pharmacological experimentation.

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Disclosure statement

The authors declare no conflict of interest.

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